

11 mRNA preparations, we designed high G-C content promoter-primers (SEQ ID. 3 and 5) annealed to the most 5'-end of a cDNA template in the sense orientation for generating full-length mRNAs using in-vitro transcription reactions (not PCR-based reactions) and therefore without the drawbacks of PCR. Because the capability of using transcriptional amplification in either the sense (mRNA) or both orientations, our invention advantageously provides more flexibility in the generation of either pure full-length mRNAs (FIG.1) or double-stranded RNA (mRNA/aRNA) mixtures (FIG.2), ready for a variety of biochemical applications such as full-length mRNA/cDNA preparation, probe preparation, in-vitro translation and gene knockout analysis (RNA interference).

Page 14, line 10, before "at 94°C", change "stooped" to --stopped--.

22 Page 15, after line 20, insert --The Example 1 describes a preferred step for the prevention of intracellular RNA degradation before the step (a) of the present invention. The Examples 2-4 are directed to each step of the second preferred embodiment (FIGS.2 and 3), while the Example 5 is draw to the first preferred embodiment (FIG.1) published in *Nucleic Acid Res.* 27: 4585-4589 (1999). Since previous methods failed to preserve the complete 5'-end of an RNA sequence for amplification due to the lack or loss of specific primer binding sites or due to the failure of the PCR reaction through polyG-C tails, our invention overcomes this bottleneck by designing special promoter-primers annealing to the 5'-added tail of a reverse-transcribed cDNA template in the sense orientation for generating full-length mRNAs using in-vitro transcription reactions (not PCR-based reactions). Although some commercialized buffering conditions for each enzymatic step component (Examples 2-4) are slightly different, we have designed a new RT&T buffer (as shown in Example 5) to unify the optimized condition for continuously thermocycling amplification of mRNAs. The changes of reacting temperatures during the thermocycling amplification is also specially designed (as shown in Examples 1-5) to fulfill the maximal activities of each enzymatic step, depending on the property of the enzyme involved. It has been proven that such unified conditions are capable of amplifying full-length mRNAs up to 5kb with reproducible results from as few as twenty cells (Lin et.al, 1999). The present invention is useful when linear amplification is required or only picogram starting materials can be acquired for mRNA/cDNA amplification. Its results not only provide molecular diagnosis of cancerous genes in vivo but also increase the resolution of current genetic research to the single-cell scale.--.